A Collaboration with the Colorectal Cancer Family Registry (CFR)

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Overview: Colon CRF Collaborations

A. Prior collaboration with CFR: a facile method to detect hMSH2 germline deletions in Lynch syndrome
B. Current collaboration: determination of the mechanism of inactivation of the wild-type allele in Lynch syndrome
C. Proposed collaboration: a search for the mechanistic basis of “Syndrome X”

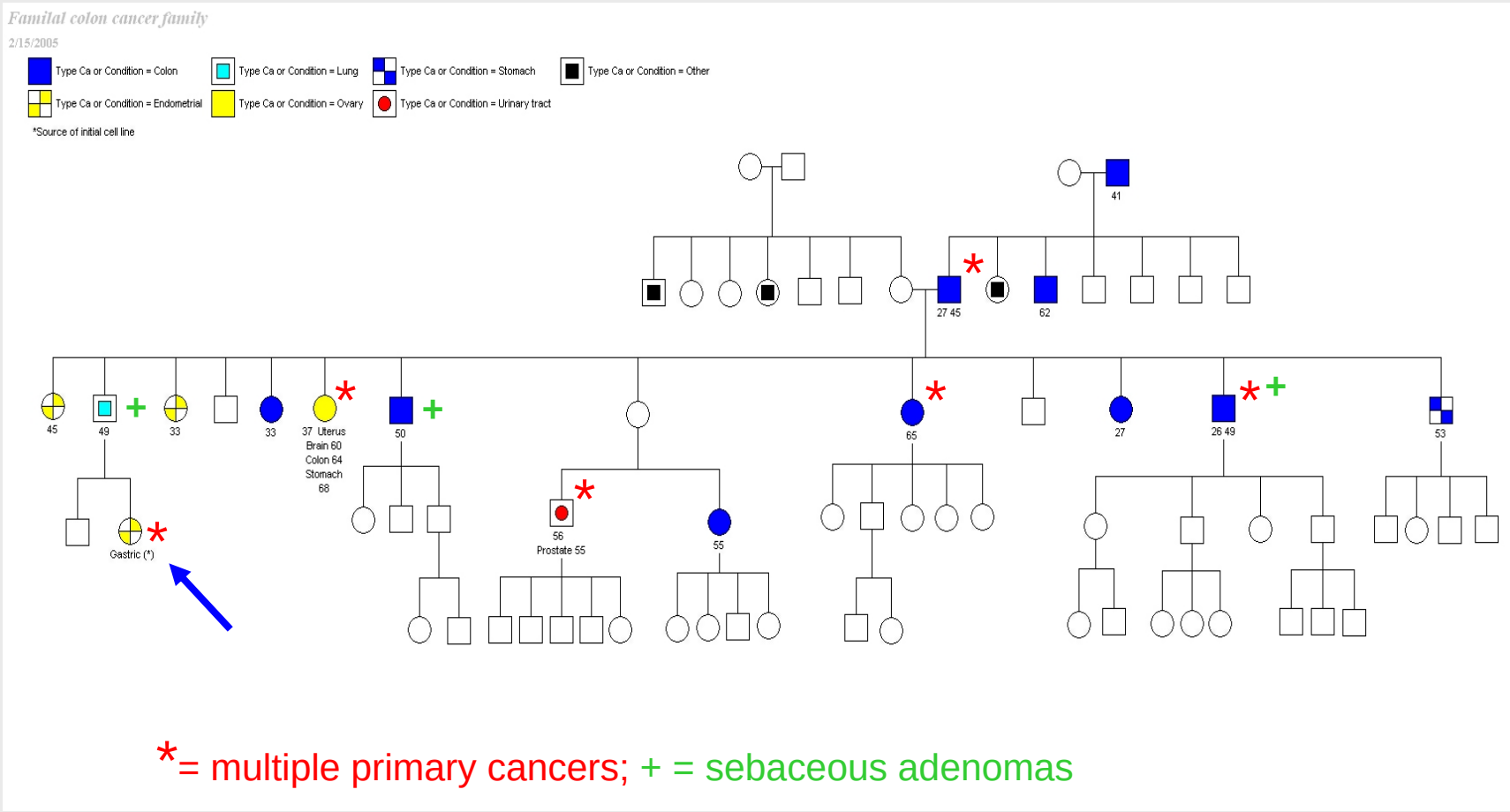
Large Deletions in hMSH2 and Lynch syndrome

CASE PRESENTATION: GT

- GT: 55 year old woman, stomach cancer
  - also has endometrial cancer, age 55
    - hysterectomy performed
  - Father died of lung cancer, age 48, was a smoker

- She died within two years of metastatic gastric cancer in spite of surgery, and other treatment

Pedigree: GT



Lynch syndrome (Muir-Torre variant)

Genetic Testing On GT

- Lynch syndrome considered on basis of family history
- Blood tested by IVTT (in vitro transcription-translation test, i.e., truncated protein test)
  - Negative
- Blood tested by direct sequencing of hMLH1 and hMSH2
  - Both “normal”, without mutation

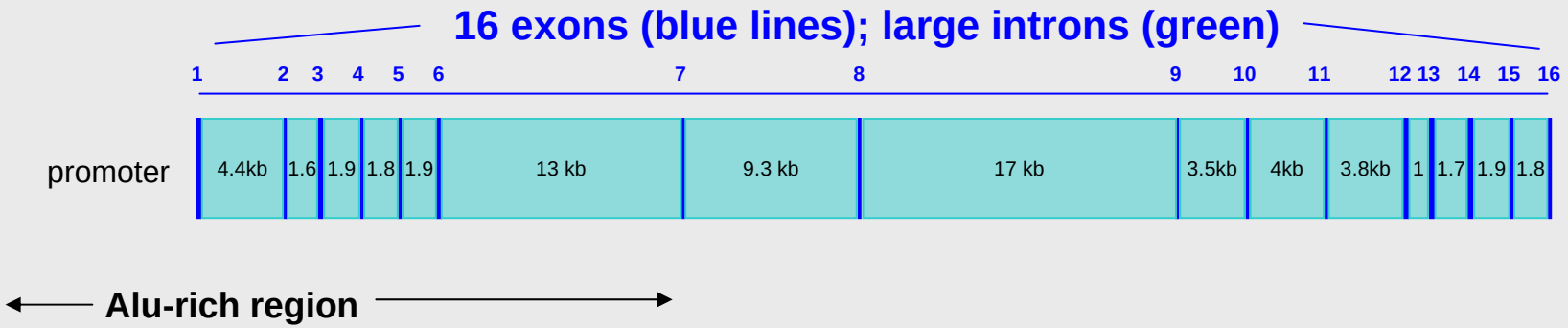
The Problem With hMSH2

- Genomic deletions of hMSH2 are a frequent cause of HNPCC
  - Make up one third of pathogenic hMSH2 mutations in a Dutch registry
    - and, 6.5% of all Amsterdam criteria positive families
  - Can be detected indirectly by Southern analysis
    - Cut DNA with restriction enzyme, separate on gels
    - Probe with multiple hMSH2 sequences in and around the gene to look for anomalously migrating band
  - These mutations are missed by conventional testing

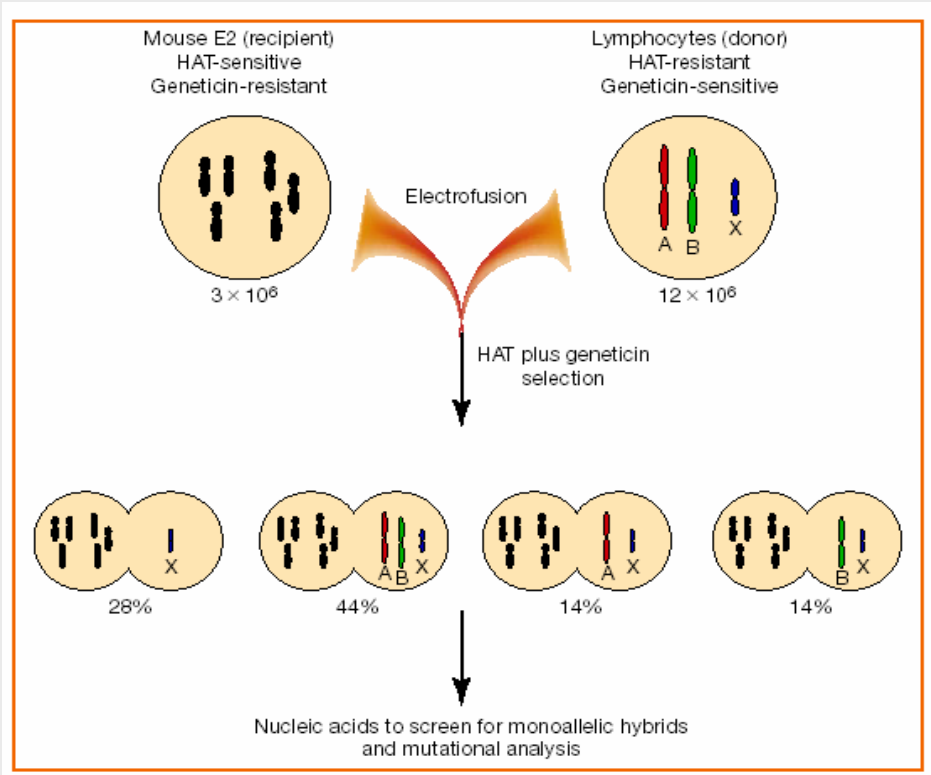
Wijnen et al, Nature Genetics 20:326, 1998

hMSH2 Gene

- **Genomic DNA = 73 KB**
- **16 exons**
- **mRNA = 2.7 kb**



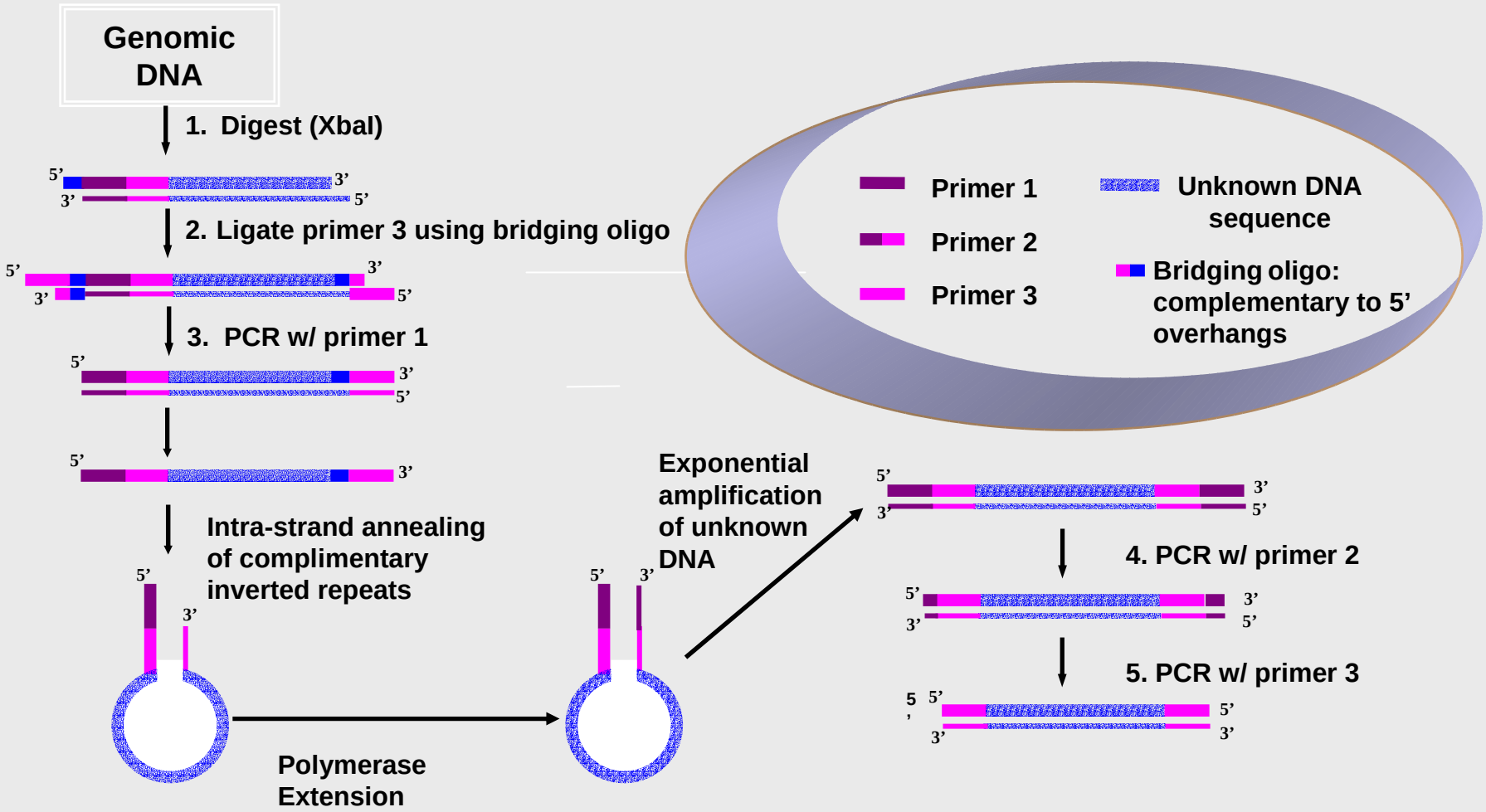
Conversion of Diploidy to Haploidy



Chromosome 2 isolated that did not express hMSH2
- Exons 1-6 deleted; 7-16 present

Yan et al. Nature, 403:7232, 2000

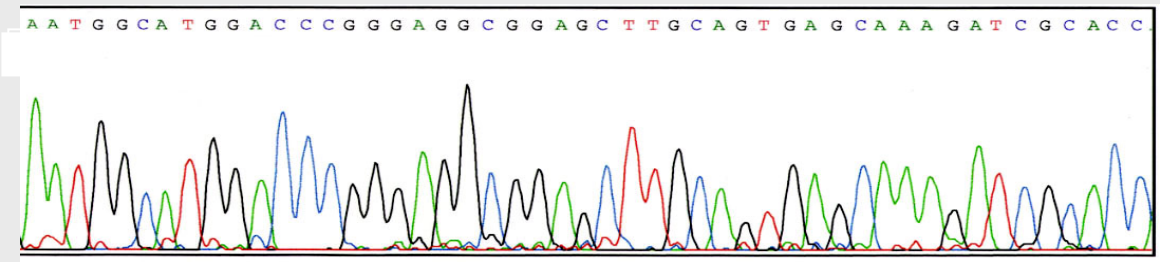
Panhandle PCR



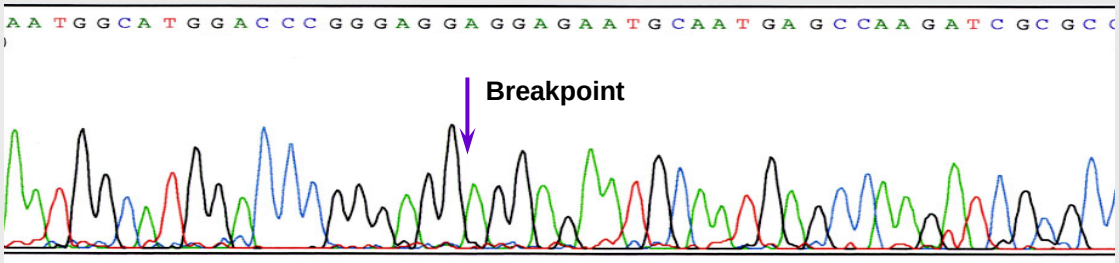
Breakpoint cloned (Rhees and Yurgelun, unpublished)

Sequencing Results

Wild type Sequence

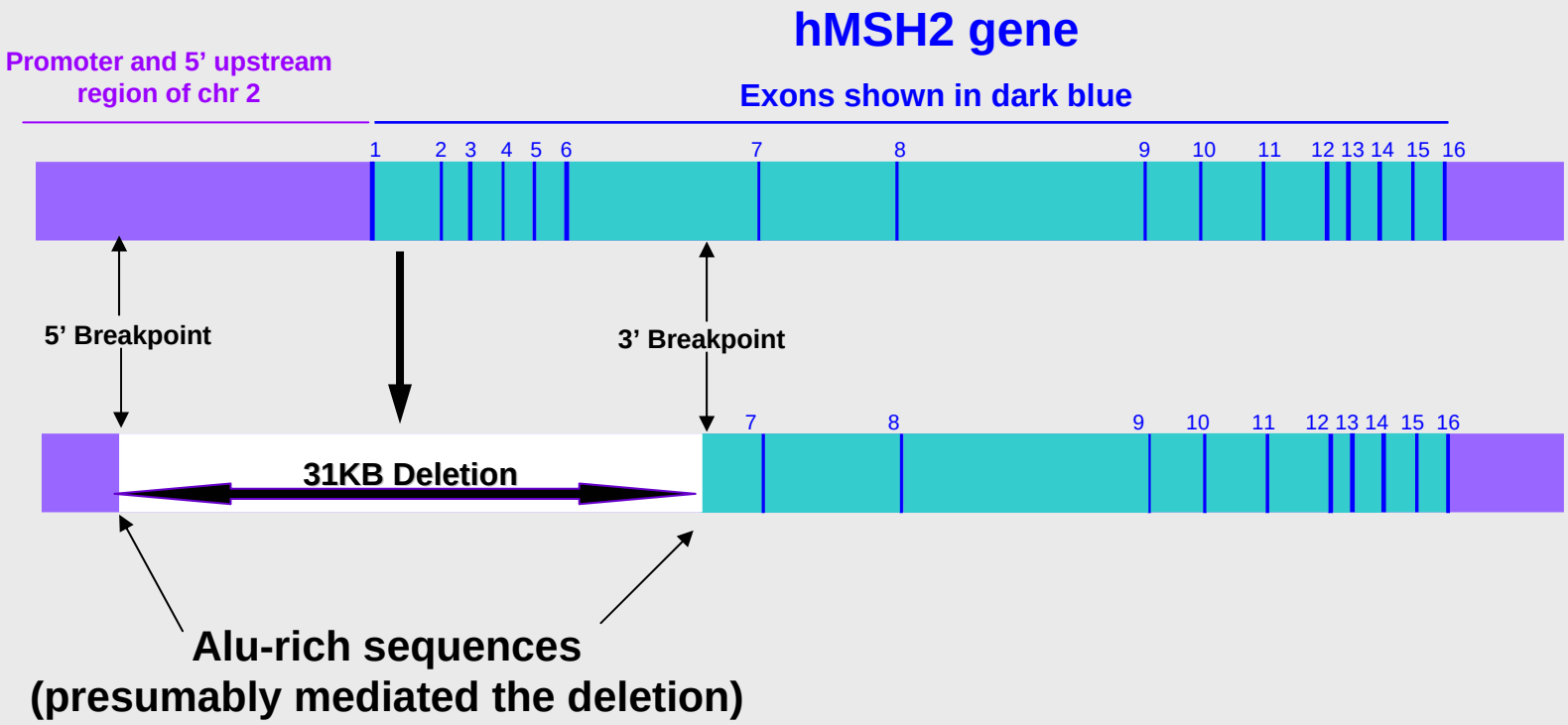


Deleted Sequence

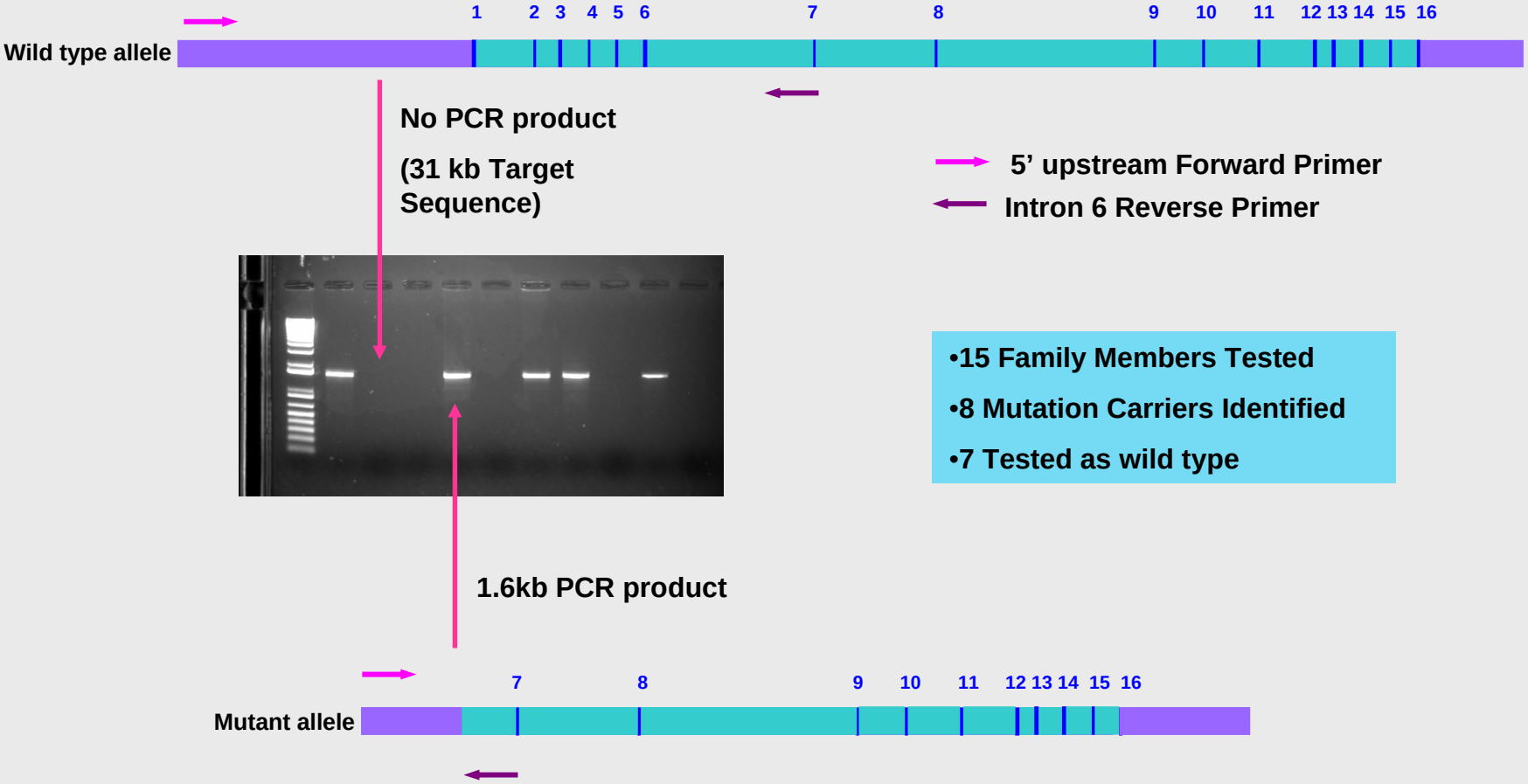


Breakpoint confirmed: flanked by Alu's

Exons 1-6 Deletion

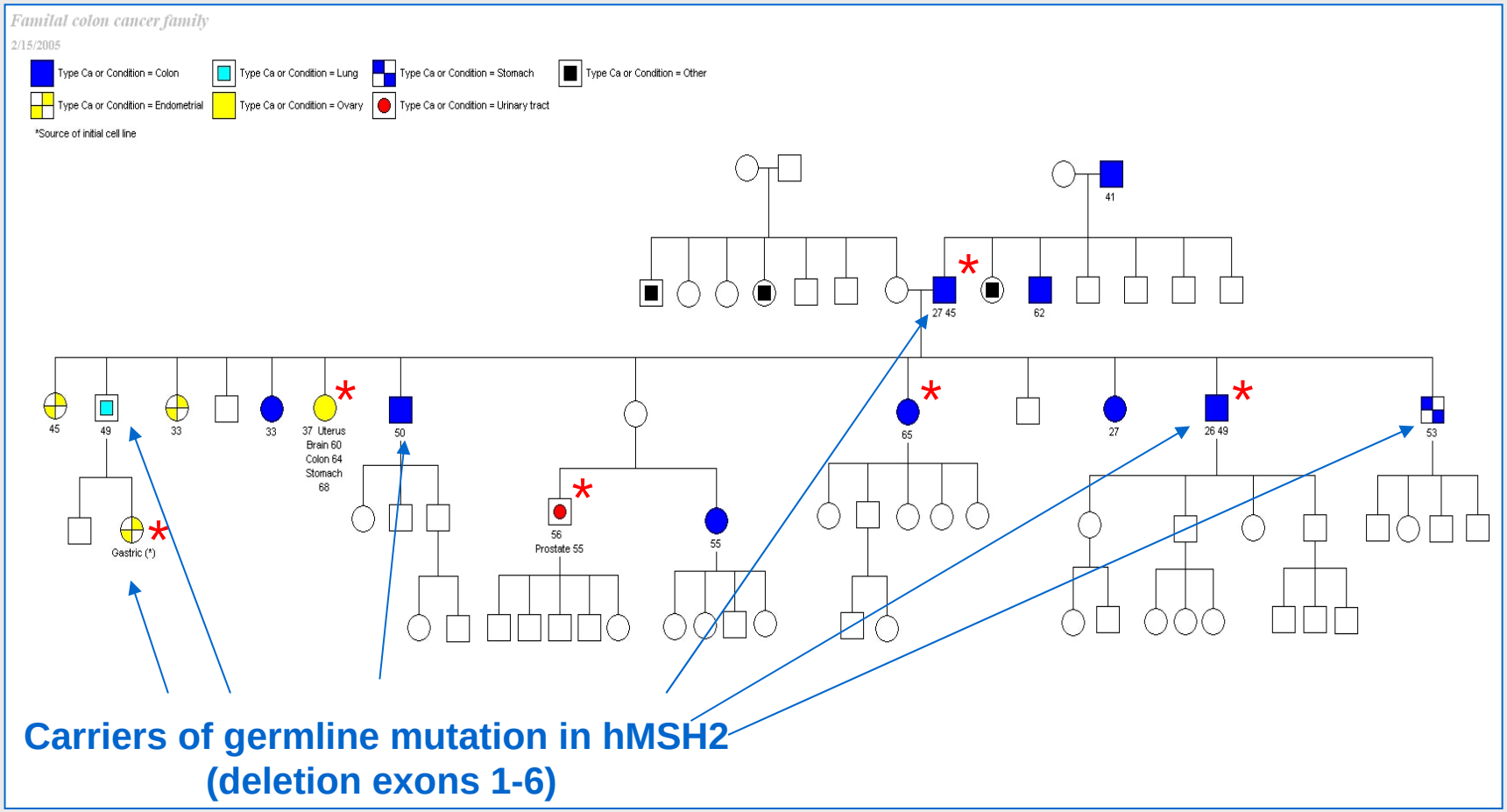


PCR Strategy and Results

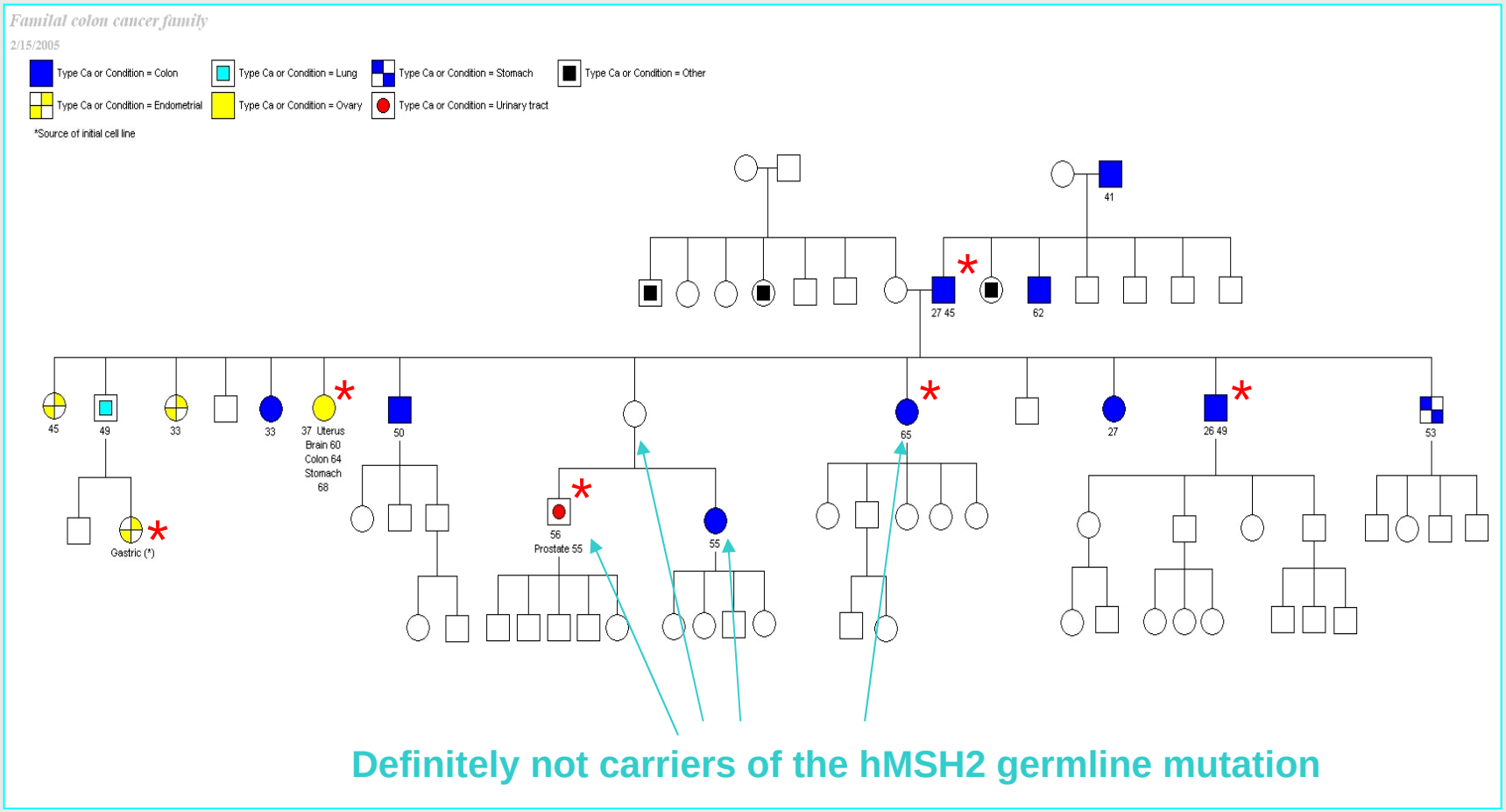


Customized PCR-based screening test developed

Carriers of the Deletion in hMSH2



Carriage of the hMSH2 deletion ruled out in 3 with cancers



A. Prior Collaboration with CFR

- Obtained samples of germline DNA from patients with deletions of DNA MMR genes
  - DFCI (10 – mutations not defined)
  - Toronto (4), USC (2): mutations defined, but none were 5’ deletions
- Mixed group: none had 5’ MSH2 deletions
- PCR strategies did not detect these deletions
- We moved on to MLPA for deletions

B. Current Colon CFR Collaboration

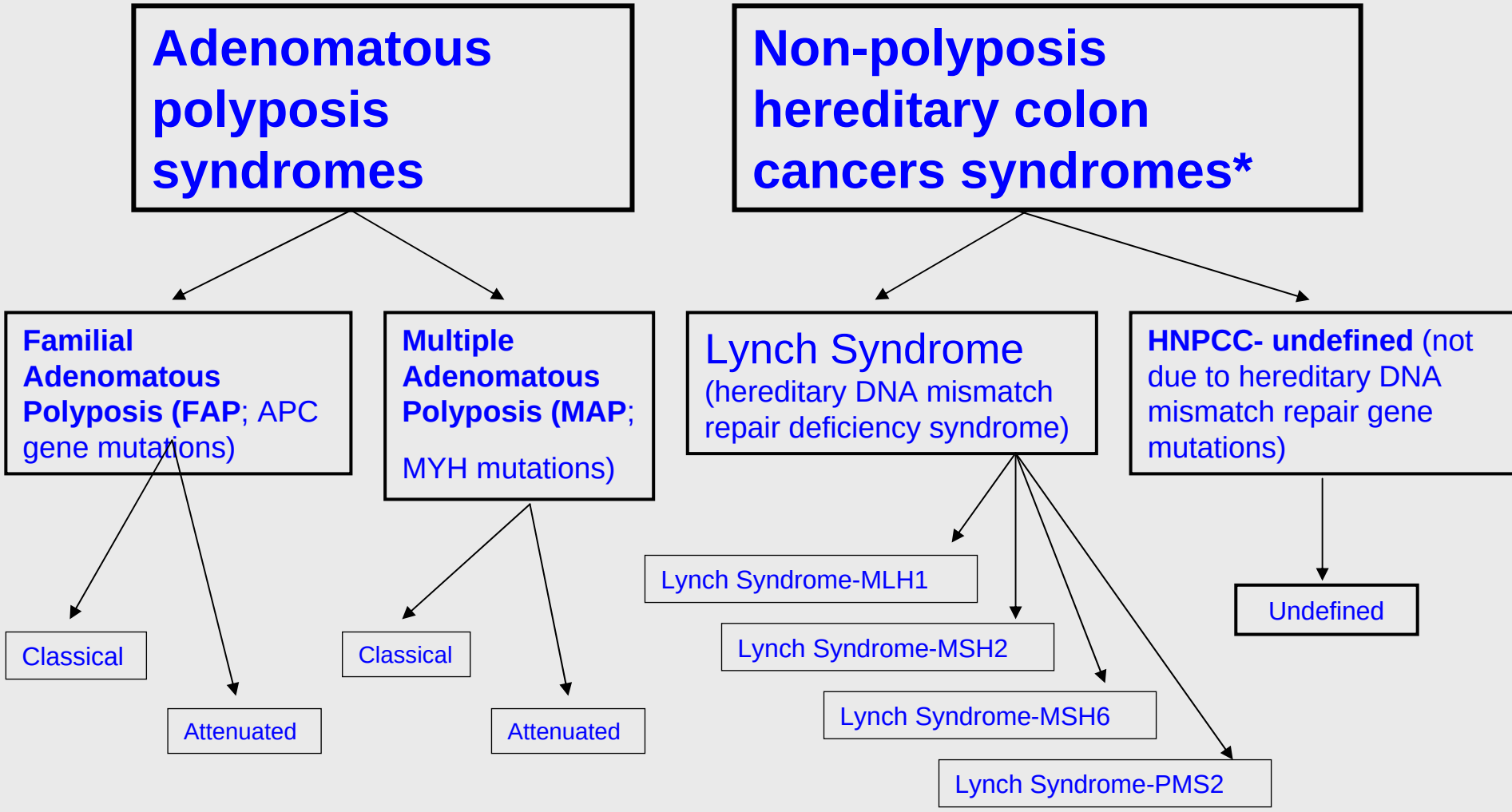
- "Second Hit" project
  - requires samples of tissue from patients with Lynch syndrome CRCs
  - germline mutation in MSH2 or MLH1 defined
  - determine the mechanism of inactivation of the wild-type allele
    - mutation, LOH, methylation
  - may assist in predicting appropriate advice for life-style modifications or chemoprevention

Progress on ‘Second Hit’ Project

- Have slides or DNA from 58 tumor specimens
  - Toronto (16), USC (19), Mayo (14), Seattle (9)
- Whole gene amplification of DNA
  - (DNA amplified from 10 ng -> 5-10 ug)
- Using dHPLC (Wave) to screen for mutations in the hMSH2 or hMLH1
  - considerable optimization has been required for WGA and dHPLC
- LOH by PCR at multiple sites per gene
  - dinucleotide repeat PCR, SNPs, MLPA
- Methylation analysis done (USC group)

C. Proposed Colon CFR Collaboration

Syndrome X project



* as defined by any of a number of pedigree criteria and/or laboratory criteria, including but not limited to the Amsterdam criteria.

Proposed Colon CFR Collaboration

- Syndrome X project
  - will require 100 tumor samples and germline DNA specimens
    - family clusters, sib-pairs
    - ? isolated young (<50 y.o.) CRC patients
  - DNA MMR mutations/MSI excluded
  - will use IHC and DNA analysis on a “candidate gene” approach
  - Productivity will be enhanced by creation of tissue microarrays (TMAs)

Experimental Approach for Syndrome X Project

Candidate Gene approach

TGFβR1 (*6A)
XRCC3
MYH
HPP1
CDX2
hPMS2
hPMS1
BLM
PTEN
Exo-1
CHK2
Axin2
DRA
BRCA1/2
Bub1/BubR1
hMLH3
hMSH3
Cyclin D*
p53*
B-RAF (*V600E)
UGT1A7*
Aurora-A (STK15)*
HRAS1 (*VNTR)
ARLTS1*
NAT2*
MTHFR*
GSTM1*

Candidate Pathway approach

MSI-L (including hMSH3 + hMLH3)
CIMP
Multiple LOH events
JCV T-Ag expression

* Indicates known polymorphism s– DNA screening by dHPLC

Methods

- Develop TMAs
- IHC for genes likely to be inactivated
- Rapid screening (WAVE) for known polymorphisms (*)
- Multiplex PCR of mono-, di-, tetranucleotide repeats for MSI-L or possible MSH3 defect
- DNA sequencing for genes found to be non-expressed at IHC